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Novel Cytotoxic Polyprenylated Xanthonoids from *Garcinia gaudichaudii* (Guttiferae)

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Abstract: Cytotoxicity guided phytochemical analysis of the leaf extract of the Malaysian medicinal plant *Garcinia gaudichaudii* led to the isolation of 15 novel cytotoxic compounds, gaudichaudiones A - H (**1**, **2**, **8** - **10** and **12**), gaudichaudiic acids A - E (**3** - **6**), including the known morellic acid (**7**) and forbesione (**11**). All are mainly tetraprenylated xanthonoids but gaudichaudione H (**12**) is a novel bridgehead methoxylated triprenylated xanthonoid. The novel caged structures were determined by detailed NMR spectral analysis and the compounds were found to exhibit significant cytotoxicity against several cancer cell lines.

INTRODUCTION

Garcinia gaudichaudii Planch et Triana is native to Indo-China, the Malay Peninsula and Borneo. The juice from the leaves of the plant is used by the natives to rub on cuts and minor wounds.[1] A preliminary communication has been made on the isolation of four cytotoxic tetraisoprenylxanthonoids[2] and the present paper reports 15 compounds of this class from the plant. Caged polyprenylated xanthonoids are elaborated by several other plants exclusively from this genus, e.g. *Garcinia morella*, *G. hanburyi* and *G. forbesii*, which have also provided various polyprenylated xanthonoids.[3-10] A common feature of the compounds is the presence of a bicyclo[2.2.2]octane or tricyclo-4-oxa[4.3.1.$0^{3,7}$]decan-2-one as part of the xanthonoid. The earliest study of the skeleton was on morellic acid,[3] the structure elucidation of which is quite challenging even with modern NMR techniques. The present plant also provided similar types of structurally complex gaudichaudiic acids in addition to gaudichaudiones, some of which were separable diastereoisomers and were elucidated by the use of 2D NMR techniques. All the compounds were also found to have significant cytotoxic potency against several cancer cell lines.

RESULTS AND DISCUSSION

Among the 15 compounds isolated from the plant were two xanthonoid-dione derivatives, gaudichaudiones E and F (**1a** and **2a**), with a skeleton similar to gaudichaudiones D and B (**1b** and **2b**), previously reported.[2] Gaudichaudione E (**1a**) was an optically active yellow oil, $[\alpha]_D^{25}$ -436.2°, and its molecular formula was determined as $C_{33}H_{38}O_8$ by HREIMS. The IR spectrum showed absorption bands at 3454 (OH), 1739 (an unconjugated carbonyl group), 1689 (an α,β-unsaturated carbonyl group) and 1635 cm^{-1} (a chelated *ortho*-hydroxyl carbonyl group). In the UV spectrum, the long wavelength absorption band (λ_{max} 356 nm, log ε 3.96) was similar to that of gambogenin (λ_{max} 362 nm, log ε 4.22).[9] The ^1H NMR (Table 1), ^{13}C NMR (Table 2), H-H COSY, NOESY and HMQC spectra of **1a** revealed the presence of one chelated hydroxyl group [δ_H 12.53 (s, OH)], one aldehyde

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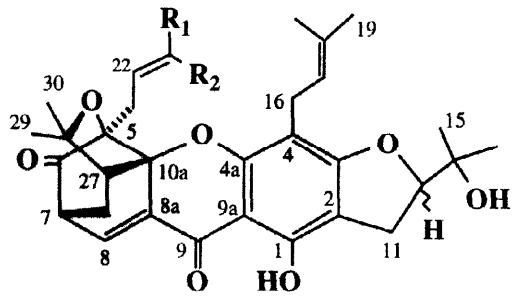
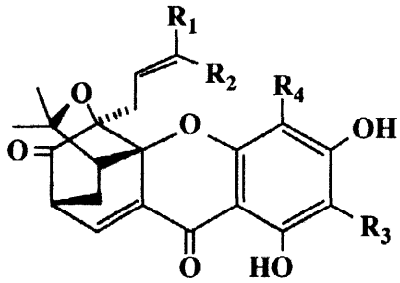
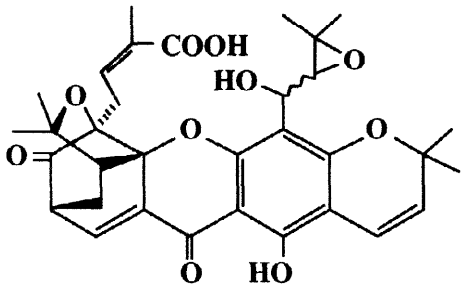


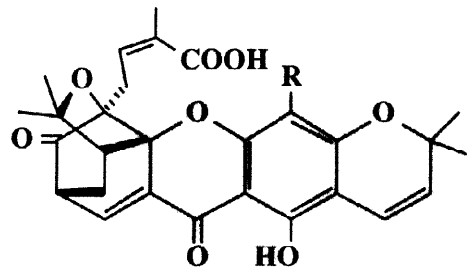
1a, 1b diastereomers : R_1 = CHO, R_2 = Me
3: R_1 = Me, R_2 = COOH



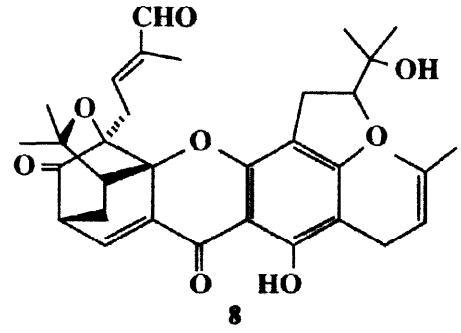
2a, 2b diastereomers : R_1 = CHO, R_2 = Me,
R_3 = 2-hydroxy-3-methylbut-3-enyl,
R_4 = 3-methylbut-2-enyl
4: R_1 = Me, R_2 = COOH, R_3 = 3-methylbut-2-enyl
R_4 = 2-hydroxy-3-methylbut-3-enyl



5a, 5b diastereomers



6: R = 2-hydroxy-3-methylbut-3-enyl
7: R = 3-methylbut-2-enyl



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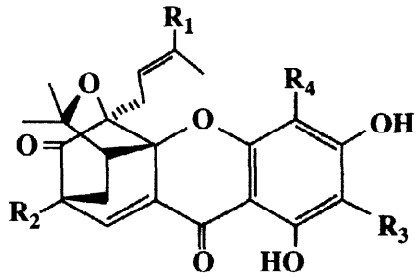


9 : R_1 = CHO, R_2 = H, R_3 = R_4 = 3-methylbut-2-enyl
10: R_1 = CHO, R_2 = H, R_3 = 3-methylbut-2-enyl,
R_4 = 2-hydroxy-3-methylbut-3-enyl
11: R_1 = Me, R_2 = R_3 = H, R_4 = 3-methylbut-2-enyl
12: R_1 = Me, R_2 = OMe, R_3 = H,
R_4 = 3-methylbut-2-enyl

Figure 1. Prenylated Xanthonoid Structures

group [δ_H 9.25 (s, H-24)], and four proton-coupled systems involving isoprenyl groups. The following structural information could be deduced: one isoprenyl group which formed a 2-(2-hydroxypropyl)dihydrofuran ring [δ_H 3.06 (dd, J = 9.7 and 15.3, Ha-11), 3.23 (dd, J = 6.4 and 15.3, Hb-11), 4.73 (dd, J = 6.4 and 9.7, H-12), 1.21 (s, H_3-14), and 1.47 (s, H_3-15)]; one 3-methylbut-2-enyl group [δ_H 3.12 (dd, J = 8.1 and 14.5, Ha-16), 2.93 (dd, J = 5.1 and 14.5, Hb-16), 5.02 (m, H-17), 1.70 (s, H_3-19), 1.64 (s, H_3-20)]; one 3-formylbut-2-enyl group [δ_H 2.79 (m, Ha-21), 2.30 (dd, J = 9.8 and 16.7, Hb-21), 6.70 (m, H-22), 9.25 (s, H-24), and 1.06 (br s, H_3-25)]; and a fourth isoprenyl group which formed a $CHCH_2CHCH{=}C$ system [δ_H 2.59 (d, J = 9.4, H-27), 2.33 (dd, J = 4.6 and 13.5, Ha-26), 1.33 (dd, J = 9.4 and 13.5, Hb-26), 3.52 (dd, J = 4.6 and 6.8, H-7), and 7.56 (d, J = 6.8, H-8)]. In the HMBC spectrum (Table 3) of **1a**, the correlations of the chelated hydroxyl group at C-1 with C-1, C-2 and C-9a (δ_C 157.5, 106.6 and 100.9), H_2-11 and H-12 with C-2 and C-3 (δ_C 106.6 and 168.6), which confirmed the presence of the pyran ring, and H_2-16 with C-3, C-4 and C-4a (δ_C 168.6, 102.8 and 158.1) indicated that one part

of the molecule was a phloroglucinol-type aromatic ring with one chelated hydroxyl group at C-1 and one isoprenyl group at C-4, which was *para* to the chelated hydroxyl group. A 2-(2-hydroxypropyl)dihydrofuran ring was fused to positions C-2 and C-3. The deshielded signal at C-1 (δ_H 12.53, s) suggested that a carbonyl group was *peri* to the hydroxyl group. These observations provided the structure of the right part of the molecule (Fig. 1) which resembled that of the xanthone morusignin G.[11] The correlations of the methine proton (δ_H 2.59, H-27) to C-5 and C-10a (δ_C 84.1 and 91.0), and of the methylene protons (δ_H 1.33 and 2.33, H_2-26) to C-6, C-8, and C-10a (δ_C 203.1, 135.4 and 91.0) confirmed that another part of **1a** contained a bicyclo[2.2.2]octane ring system. The chemical shifts of C-28 and C-5 which appeared at δ_C 84.2 and 84.1 respectively revealed that there was an ether linkage between these two carbons, neither of which was linked with C-4a, otherwise they should appear at about 90 ppm, e.g. C-10a. Furthermore, if the *O*-linkage at C-5 and C-28 were hydroxyl groups, their ^{13}C NMR chemical shifts (C-5 and C-28) should be close to 70 ppm.[12] Hence, another partial structure of **1a** was a rare tricyclo-4-oxa[4.3.1.$0^{3,7}$]decan-2-one system.[13] The methylene protons (δ_H 2.30 and 2.79, H_2-21) of the 3-formybut-2-enyl group correlated with C-10a and C-5 (δ_C 91.0 and 84.1), which enabled its placement at C-5. The configuration at the C-22 double bond could be determined as *E* from NOESY correlations. This was further confirmed by the relatively deshielded aldehyde group [δ_H 9.25 and δ_C 195.7 (values of δ_H 9.0, and δ_C 190 are expected for a *Z* configuration)].[9] The structure of the left (caged) fragment was determined as shown. The correlation between H_3-25 and H_2-16 in the NOESY spectrum (Fig. 2) allowed the assignment of the structure of gaudichaudione E as a diastereoisomer **1a** (arising from the introduction of another chiral centre at C-12) of gaudichaudione D (**1b**) which was described earlier.[2]

Gaudichaudione F (**2a**) was also isolated as a yellow oil. Based on the HREIMS (*m/z* 562.2574), the optically active compound, $[\alpha]_D^{25}$ -469.6°, had the molecular formula $C_{33}H_{38}O_8$ isomeric to **1a**. The tricyclo-4-oxa[4.3.1.$0^{3,7}$]decan-2-one moiety of **2a** was identical to that of **1a**, but the substituents at C-2 (2-hydroxy-3-methylbut-3-enyl) and C-3 (OH) on the phloroglucinol-type ring of **2a** differed from **1a**. Detailed comparison of the data [UV, IR, NMR (Tables 1 and 2), HMQC and HMBC (Table 3)] for compound **2a** with others[2] (Fig. 1) revealed that it was a diastereoisomer of gaudichaudione B (**2b**).[2]

Gaudichaudiic acid A (**3**), $[\alpha]_D^{25}$ -281.7° (*c* 3.41, $CHCl_3$), isolated as a yellow oil, was found to have the molecular formula $C_{33}H_{38}O_9$ by HREIMS. The IR spectrum of the compound exhibited absorption bands at 3443 (OH), 1736 (an unconjugated carbonyl group), 1665 (an α,β-unsaturated carbonyl group) and 1625 (a chelated *ortho*-hydroxyl carbonyl group) cm^{-1}. In the UV spectrum, the long wavelength absorption band (λ_{max} 354 nm, log ε 4.05) was similar to that of **1a**. The ^{1}H NMR (Table 1), ^{13}C NMR (Table 2), H-H COSY, NOESY and HMQC spectra of **3a** indicated the presence of one chelated hydroxyl group [δ_H 12.48 (s, OH)] and four proton-coupled systems involving isoprenyl groups. There were one 2-(2-hydroxypropyl)dihydrofuran ring [δ_H3.09 (m, H_2-11), 4.73 (dd, *J* = 6.5 and 9.1, H-12), 1.24 (s, H_3-14), and 1.46 (s, H_3-15)], one 3-methylbut-2-enyl group [δ_H 3.29 (m, H_2-16), 5.20 (m, H-17), 1.74 (s, H_3-19), 1.68 (s, H_3-20)], one 3-carboxylbut-2-enyl group [δ_H 2.73 (m, Ha-21), 3.52 (m, Hb-21), 5.41 (m, H-22), and 1.63 (s, H_3-25)], and a further isoprenyl group which formed a CH-CH_2-CH-CH=C system [δ_H 2.56 (d, *J* = 9.3, H-27), 2.33 (dd, *J* = 4.6 and 13.4, Ha-26), 1.33 (dd, *J* = 9.3 and 13.4, Hb-26), 3.52 (dd, *J* = 4.6 and 6.8, H-7), and 7.45 (d, *J* = 6.8, H-8)]. HMBC (Table 3) allowed the assignment of one part as a diprenylated phloroglucinol-type aromatic ring and another part possessing the tricyclo-4-oxa[4.3.1.$0^{3,7}$]decan-2-one system, similar to **1a**. The only difference between **3** and **1a** was the interchange of a carboxylic acid group with the aldehyde group. The configuration of the double bond (at C-22) also differed and was determined to be in the *Z* configuration by virtue of the chemical shifts of the vinyl proton H-22 (δ_H 5.41) and the tertiary methyl carbon (δ_C 20.9, C-25); otherwise, if the double bond was in the *E* configuration, the chemical shift would be observed at 11-14 ppm (δ_C) for the methyl carbon (C-25).[14] The observation of a cross-peak in the NOESY spectrum between H-22 and Me-25 further verified this deduction. The placement of the prenyl group in position 4 rather than 2 was established by correlations in the NOESY spectrum (Fig. 2) of H_2-16 and H-17 to Me-25. Consequently, the structure of gaudichaudiic acid A could be assigned as **3**.

Gaudichaudiic acid B (**4**) was also isolated as a yellow oil. Based on the HREIMS (*m/z* 578.2518), the optically active compound, $[\alpha]_D^{25}$ -325.5°, had the molecular formula $C_{33}H_{38}O_8$, and was an isomer of **3**. From the NMR data the tricyclo-4-oxa[4.3.1.$0^{3,7}$]decan-2-one moiety of **4** was determined to be identical to that of **3**, but the substituents at C-2 (3-methylbut-2-enyl), C-3 (OH) and C-4 (2-hydroxy-3-methylbut-3-enyl) on

Table 1. ^{1}H NMR Data of Compounds **1 - 4** (in $CDCl_3$)*

C#	**1a**	**2a**	**3**	**4**
7	3.52 (1H, dd, 4.6, 6.8)	3.52 (1H, dd, 4.7, 6.8)	3.52 (1H, dd, 4.6, 6.8)	3.50 (1H, dd, 4.4, 6.8)
8	7.56 (1H, d, 6.8)	7.54 (1H, d, 6.8)	7.45 (1H, d, 6.8)	7.53 (1H, d, 6.8)
11	3.23 (1H, dd, 6.4, 15.3)	2.95 (2H, m)	3.09 (2H, m)	3.26 (2H, d, 7.0)
	3.06 (1H, dd, 9.7, 15.3)			
12	4.73 (1H, dd, 9.7, 6.4)	4.40 (1H, m)	4.73 (1H, dd, 6.5, 9.1)	5.20 (1H, t, 7.0)
14	1.21 (3H, s)	4.88 (1H, br s)	1.24 (3H, s)	1.67 (3H, s)
		4.95 (1H, br s)		
15	1.47 (3H, s)	1.83 (3H, s)	1.46 (3H, s)	1.75 (3H, s)
16	3.12 (1H, dd, 8.1, 14.5)	3.32 (2H, m)	3.29 (2H, m)	2.75 (1H, dd, 9.5, 13.5)
	2.93 (1H, dd, 5.1, 14.5)			3.09 (1H, d, 13.5)
17	5.02 (1H, m)	5.14 (1H, m)	5.20 (1H, m)	4.28 (1H, d, 9.5)
19	1.70 (3H, s)	1.74 (3H, s)	1.74 (3H, s)	4.88 (1H, br s)
				4.89 (1H, br s)
20	1.64 (3H, s)	1.67 (3H, s)	1.68 (3H, s)	1.86 (3H, s)
21	2.30 (1H, dd, 9.8, 16.7)	2.71 (2H, m)	2.73 (2H, m)	2.83 (1H, dd, 7.0, 15.8)
	2.79 (1H, m)		3.52 (1H, m)	3.20 (1H, dd, 7.0, 15.8)
22	6.70 (1H, m)	6.30 (1H, t, 6.8)	5.41 (1H, m)	5.72 (1H, t, 7.0)
24	9.25 (1H, s)	9.18 (1H, s)		
25	1.06 (3H, br s)	1.25 (3H, s)	1.63 (3H, s)	1.70 (3H, s)
26	1.33 (1H, dd, 9.4, 13.5)	1.40 (1H, dd, 9.4, 13.5)	1.33 (1H, dd, 9.3, 13.4)	1.38 (1H, dd, 9.3, 13.5)
	2.33 (1H, dd, 4.6, 13.5)	2.36 (1H, dd, 4.7, 13.5)	2.33 (1H, dd, 4.6, 13.4)	2.31 (1H, dd, 4.4, 13.5)
27	2.59 (1H, d, 9.4)	2.55 (1H, d, 9.4)	2.56 (1H, d, 9.3)	2.46 (1H, d, 9.3)
29	1.32 (3H, s)	1.32 (3H, s)	1.28 (3H, s)	1.26 (3H, s)
30	1.72 (3H, s)	1.74 (3H, s)	1.67 (3H, s)	1.58 (3H, s)
1-OH	12.53	12.88	12.48	12.79

* Chemical shifts in ppm and in parentheses (no. of protons, multiplicity, coupling constants in Hz)

Table 2. ^{13}C NMR Chemical Shifts for Compounds **1 - 4, 8, 12** (in $CDCl_3$)

C	**1a**	**2a**	**3**	**4**	**8**	**12**	C#	**1a**	**2a**	**3**	**4**	**8**	**12**
1	157.7	161.2	157.4	161.3	163.3	163.3	15	26.3	18.7	26.9	17.8	17.8	25.8
2	106.6	106.0	105.9	110.6	105.5	97.2	16	22.5	22.2	22.5	29.0	27.0	29.1
3	168.6	165.3	168.1	164.7	167.9	164.4	17	121.3	122.3	121.9	78.6	91.3	117.6
4	102.8	108.2	103.9	104.6	103.6	105.8	18	132.8	132.5	132.2	146.2	71.6	135.5
4a	158.1	156.3	158.5	156.1	152.9	158.1	19	17.9	18.1	18.0	113.6	24.5	16.8
5	84.1	83.5	83.5	83.7	84.2	84.3	20	25.8	25.7	25.7	16.8	26.1	25.7
6	203.1	203.2	204.1	203.2	202.5	201.8	21	28.5	29.1	29.3	29.5	28.9	30.4
7	46.6	46.9	47.2	46.9	46.6	84.9	22	147.5	146.4	136.9	136.4	147.3	49.8
8	135.4	135.3	134.5	134.9	135.7	134.5	23	139.3	140.2	128.3	128.9	139.9	83.6
8a	133.7	133.9	134.3	133.5	133.5	131.9	24	195.7	194.5	168.3	170.0	194.4	29.0
9	178.7	178.6	179.6	179.0	178.1	179.1	25	8.4	8.6	20.9	20.7	8.8	30.3
9a	100.9	100.4	101.7	100.7	100.8	101.3	26	24.8	25.3	25.2	25.3	24.9	
10a	91.0	90.5	90.4	90.5	90.9	89.4	27	49.0	49.1	48.8	49.1	48.8	
11	26.4	27.9	26.3	21.4	21.4	22.2	28	84.2	84.0	84.4	83.9	83.3	
12	91.9	77.2	90.6	122.3	121.5	121.1	29	28.8	29.0	28.9	28.8	28.9	
13	71.6	146.3	73.5	132.2	132.1	135.7	30	29.8	29.9	29.9	30.8	30.0	
14	24.3	110.3	24.1	25.8	25.7	18.1	OMe						54.0

the phloroglucinol-type aromatic ring were different from those of **3**. Detailed comparison of the data [UV, IR, NMR (Tables 1 and 2), HMQC and HMBC (Table 3)] for compound **4** with those of **3** revealed that **4** was a *seco* derivative of **3**.

Gaudichaudiic acid C (**5a**), $[\alpha]_D^{25}$ -355.7°, isolated as a yellow oil, was found to have the molecular formula $C_{33}H_{36}O_{10}$ by HREIMS. ^1H NMR and HMQC spectra indicated the presence of four coupled systems involving isoprenyl groups. The first coupled system was an isoprenyl group which formed a dimethylpyran ring [δ_H 6.63 (d, J = 10.0 Hz, H-11), 5.55 (d, J = 10.0 Hz, H-12), and two tertiary methyl groups at δ_H 1.55 and 1.48]. The second group was a 1-hydroxy-2,3-epoxy-3-methylbutyl group [δ_H 5.18 (d, J = 4.1 Hz, H-16), 3.33 (d, J = 4.1 Hz, H-17), 1.31 (s, Me-19), 1.30 (s, Me-20)].[15] The third system, a 3-carboxylbut-2-enyl group [δ_H 3.43 (dd, J = 10.5, 14.7 Hz, Ha-21), 2.93 (m, Hb-21), 5.64 (m, H-22), 1.68 (s, Me-25)] was identical to that of **3** and **4**, while the fourth group was a $CHCH_2CHCH{=}C$ system [δ_H 7.57 (d, J = 7.0 Hz, H-8), 3.53 (dd, J = 4.6, 7.0 Hz, H-7), 2.48 (d, J = 9.3 Hz, H-27), 2.36 (dd, J = 4.6, 13.6 Hz, Ha-26), 1.37 (dd, J = 9.3, 13.6 Hz, Hb-26)]. Correlations in the HMBC spectrum (Table 3) of the chelated hydroxyl proton at C-1, the olefinic protons H-11 and H-12, and also the methine proton H-16 to the corresponding carbons showed that the chelated hydroxyl, dimethylpyran and 1-hydroxy-2,3-epoxy-3-methylbutyl groups were all attached to the same phloroglucinol-type aromatic ring, with the chelated hydroxyl group *para* to the 1-hydroxy-2,3-epoxy-3-methylbutyl group. Furthermore, the deshielded nature of the C-1 hydroxyl proton (δ_H 12.99) meant that a carbonyl group had to be *peri* to the hydroxyl group in question. This allowed the formulation of the partial structure, i.e. the right fragment of the **5a** molecule (Fig. 1). Further information obtained from the HMBC spectrum allowed the location of the $CHCH_2CHCH{=}C$ group identified earlier. Correlations in the NOESY spectrum from H-16 and H-17 to Me-25 implied that the 3-carboxyl-but-2-enyl group was attached to C-5. The double bond at C-22 was also determined to be in the *Z* configuration as for **3**. Therefore, the left fragment with structure as shown as **5a** (Fig. 1), could be deduced as being the same as that of **3**. Finally, correlations in the NOESY spectrum (Fig. 2) from H-16 and H-17 to Me-25 implied that one bond linking C-9a to C-9 and another from C-4a to the oxygen attached to C-10a would result in the required xanthonoid skeleton. The preceding spectral considerations thus allowed the NMR assignment (Tables 4 and 5) of structure **5a** to gaudichaudiic acid C.

Compound **5b**, $[\alpha]_D$ -254.3°, was also isolated as a yellow oil, and HREIMS indicated that compounds **5b** and **5a** had the same molecular formula. The ^1H and ^{13}C NMR spectra of **5b** were almost (but not exactly) identical to those of **5a**, albeit for some minor differences. The same four coupled isoprenyl systems found in **5a**, *viz.* the dimethylpyran ring, the 3-carboxylbut-2-enyl group, the 1-hydroxy-2,3-epoxy-3-methylbutyl group and the $CHCH_2CHCH{=}C$ system were also present in **5b**, and were attached to the same carbon positions as in compound **5a**. Since both compounds were structurally similar, but exhibited subtle differences in the ^1H and ^{13}C NMR spectra gave (Tables 4 and 5) and were separable by normal SiO_2 TLC, it was concluded that they were diastereomers and **5b** was duly named gaudichaudiic acid D.

Compound **6**, $[\alpha]_D$ -160.1°, was isolated as a yellow oil. HREIMS determined the molecular ion as $C_{33}H_{36}O_9$, one oxygen less than compounds **5a** and **5b**. A comparison of the ^1H and ^{13}C NMR spectra (Tables 4 and 5) of **6** with **5a** showed that the substituent at C-4 in **6** was different from the corresponding one in **5a**. There was a 2-hydroxy-3-methylbut-3-enyl group in compound **6**, which gave rise to a set of new signals, most noticeably two new singlets in the ^1H NMR spectrum at δ_H 4.97 and 4.85 ppm, typical of a pair of olefinic methylene protons on a terminal double bond. The observation of signals at δ_C 147.4, 110.9 and 17.9 ppm (C-18, C-19 and C-20 respectively) was also characteristic of a $CH_2{=}C(CH_3)$ moiety. With the above data gaudichaudiic acid E (**6**) was assigned as shown. This compound is structurally related to the known morellic acid (**7**)[3] but with unknown absolute stereochemistry.

Gaudichaudione G (**8**) was isolated as an optically active yellow oil, $[\alpha]_D^{25}$ -197.9°, and its molecular formula was determined as $C_{33}H_{38}O_8$ by high resolution EIMS. The major characteristic bands in the IR and UV spectra were similar to gaudichaudiones A, B, E and F (**9**, **2b**, **1a** and **2a**) described above. The ^1H NMR (Table 4), ^{13}C NMR (Table 2), ^1H-^1H COSY, NOESY and HMQC spectra revealed the presence of one 2-(2-hydroxypropyl)-dihydrofuran ring [δ_H 2.58 (dd, J = 9.0 and 16.5, Ha-16), 2.68 (dd, J = 6.9 and 16.5, Hb-16), 4.67 (dd, J = 6.9 and 9.0, H-17), 1.23 (s, H_3-19), and 1.36 (s, H_3-20)] attached to C-3,4 and one 3-methylbut-2-enyl

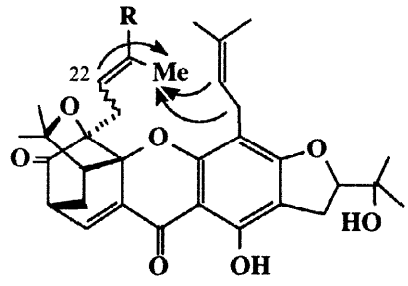
Table 3. Major long range correlations for **1a**, **2a**, **3**, **4**, **5a** and **8** in the HMBC experiments

Proton	^2J correlation	^3J correlation	Notes
H-7	C-6[d], C-8[a]	C-5[ab], C-8a[abc], C-27	not observed in **5a** and **8**
H-8	C-7	C-6[e], C-9, C-10a	
H-11	C-2[e], C-12[ae]	C-1[ae], C-3, C-13	
H-12	C-13[abd]	C-2[df], C-3[bdef], C-14/15[ace]	
H-14	C-13	C-12, C-15	
H-15	C-13	C-12, C-14	
H-16	C-4[e], C-17	C-3, C-4a[e], C-18[e]	
H-17	C-16[ade]	C-19, C-20, C-4[abe]	not observed in **5a** and **8** (except C-3)
H-19	C-18	C-17[e], C-20	
H-20	C-18	C-17, C-19	
H-21	C-5, C-22	C-10a, C-23, C-6[ae]	not observed in **5a** except C-22
H-22	C-21[acde], C-23[abde]	C-24, C-25, C-5[ade]	not observed in **5a** and **8** (except C-24 & C-25)
H-24	C-23	C-22, C-25	not observed in **3**, **4** & **5a**
H-25	C-23	C-22, C-24	
H-26	C-27, C-7[ae]	C-6, C-8, C-10a, C-28	
H-27	C-10a, C-26[def], C-28[c]	C-5[b], C-29, C-30, C-7[ade]	not observed in **5a** except C-7,10a & 28
H-29	C-28	C-27, C-30	
H-30	C-28	C-27, C-29	
1-OH	C-1	C-2, C-9a	

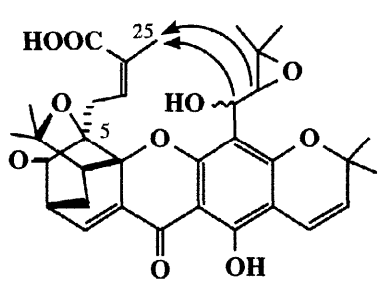
[abcdef]superscripts refer to **1a**, **2a**, **3**, **4**, **5a** and **8** respectively, where correlations were weak or not observed under the experimental conditions.

group [δ_H 3.22 (m, H_2-11), 5.21 (m, H-12), 1.69 (s, H_3-14), 1.75 (s, H_3-15)] attached to C-2. The HMBC spectrum (Table 3) of **8** supported the structure assigned, especially the correlations of H_2-16 and H-17 with C-4 (δ_C 103.6) and C-3, which confirmed the presence of the hydrofuran ring as a structural variation in the series of gaudichaudiones.

Gaudichaudione derivatives **1b**, **2b**, **9** and **10** have been reported in the earlier communication[2] but a novel 7-methoxyl derivative, gaudichaudione H (**12**) was isolated as a minor compound. **12** was a light yellow oil, $[\alpha]_D^{25}$ -132.8°, and its molecular formula was determined as $C_{29}H_{34}O_7$ by HREIMS. The IR spectrum of the compound showed absorption bands at 3454 (OH), 1744 (C=O), and 1653 cm^{-1} (chelated C=O). In the UV spectrum, the long wavelength absorption band (λ_{max} 358 nm, log ε 3.67) was similar to that of the other gaudichaudiones (e.g. **1a** and **2a**). The NMR data (Tables 4 and 2) revealed two clear features - one methoxyl [δ_H 3.63 (s, OMe-7), δ_C 54.0] at C-7 and the C-2 position was unsubstituted [δ_H 6.05 (s, H-2), δ_C 97.2 (d)]. Other features were two hydroxyl groups [δ_H 12.53 (s, OH-1, chelated) and 6.25 (s, OH-3)] and three proton-coupled systems arising from isoprenyl groups. There were two 3-methylbut-2-enyl groups [δ_H 3.40 (br. d, 6.7, H_2-11),



1a : 22 *E*, R = CHO
3 : 22 Z, R = COOH



5a & 5b

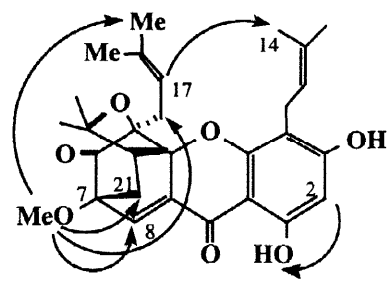


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Figure 2. NOESY and NOE-Difference Correlations

Table 4. ^{1}H NMR Data of Compounds **5a**, **5b**, **6**, **8**, and **12** (in $CDCl_3$)

C#	5a	5b	6	8	12
2					6.05 (1H, s)
7	3.53 (1H, dd, 4.6, 7.0)	3.51 (1H, dd, 4.8, 7.0)	3.50 (1H, dd, 4.7, 6.9)	3.53 (1H, dd, 4.5, 7.0)	
8	7.57 (1H, d, 7.0)	7.58 (1H, d, 7.0)	7.56 (1H, d, 6.9)	7.56 (1H, d, 7.0)	7.49 (s)
11	6.63 (1H, d, 10.0)	6.63 (1H, d, 10.0)	6.64 (1H, d, 10.0)	3.22 (2H, m)	3.40 (2H, br d, 6.7)
12	5.55 (1H, d, 10.0)	5.55 (1H, d, 10.0)	5.52 (1H, d, 10.0)	5.21 (1H, m)	5.23 (1H, m)
14	1.55 (3H, s)	1.52 (3H, s)	1.49 (3H, s)	1.69 (3H, s)	1.80 (3H, s)
15	1.48 (3H, s)	1.36 (3H, s)	1.26 (3H, s)	1.75 (3H, s)	1.75 (3H, s)
16	5.18 (1H, d, 4.1)	5.12 (1H, d, 4.9)	2.81 (1H, dd, 3.7, 14.1); 2.94 (1H, m)	2.58 (1H, dd, 9.0, 16.5); 2.68 (1H, dd, 6.9, 16.5)	2.55 (2H, m)
17	3.33 (1H, d, 4.1)	3.28 (1H, d, 4.9)	4.35 (1H, dd, 3.7, 10.2)	4.67 (1H, dd, 6.9, 9.0)	4.43 (1H, m)
19	1.31 (3H, s)	1.51 (3H, s)	4.97 (1H, s) 4.85 (1H, s)	1.23 (3H, s)	1.05 (3H, s)
20	1.30 (3H, s)	1.45 (3H, s)	1.85 (3H, s)	1.36 (3H, s)	1.38 (3H, s)
21	3.43 (1H, dd, 10.5, 14.7); 2.93 (1H, m)	2.88 (2H, m)	2.94 (1H, m); 3.07 (1H, dd, 8.3, 15.0)	2.91 (1H, dd, 9.1, 15.2); 3.03 (1H, dd, 8.5, 15.2)	1.60 (1H, dd, 9.6, 12.8); 2.36 (1H, d, 12.8)
22	5.64 (1H, m)	5.87 (1H, m)	5.86 (1H, tm, 8.3, 1.3)	6.58 (1H, dd, 8.5, 9.1)	2.60 (1H, br d, 9.6)
24				9.32 (1H, s)	1.31 (3H, s)
25	1.68 (3H, s)	1.79 (3H, s)	1.74 (3H, s)	1.33 (3H, s)	1.67 (3H, s)
26	1.37 (1H, dd, 9.3, 13.6); 2.36 (1H, dd, 4.6, 13.6)	1.38 (1H, dd, 9.4, 13.4); 2.33 (1H, dd, 4.8, 13.4)	1.38 (1H, dd, 9.3, 13.6); 2.33 (1H, dd, 4.7, 13.6)	1.40 (1H, dd, 9.8, 13.5); 2.34 (1H, dd, 4.5, 13.5)	
27	2.48 (1H, d, 9.3)	2.60 (1H, d, 9.4)	2.52 (1H, d, 9.3)	2.50 (1H, d, 9.8)	
29	1.29 (3H, s)	1.28 (3H, s)	1.29 (3H, s)	1.32 (3H, s)	
30	1.67 (3H, s)	1.73 (3H, s)	1.72 (3H, s)	1.70 (3H, s)	
1-OH	12.99	12.99	12.91	12.87	12.51 (s)
OMe					3.63 (3H, s)

* Chemical shifts in ppm and in parenthesis (no. of protons, multiplicity, coupling constants in Hz)

Table 5. ^{13}C NMR Chemical Shifts for Compounds **5a**, **5b** and **6** (in $CDCl_3$)

C#	5a	5b	6	C#	5a	5b	6	C#	5a	5b	6
1	159.5	159.4	158.3	9a	100.2	100.5	100.9	20	24.9	25.0	17.9
2	103.8	103.7	103.1	10a	91.2	91.1	91.1	21	29.9	29.7	29.5
3	161.5	161.6	161.8	11	115.1	115.1	115.4	22	135.5	136.0	135.5
4	108.3	108.5	104.8	12	126.4	126.5	125.9	23	129.8	129.4	128.7
4a	157.1	157.5	158.2	13	79.7	79.7	79.4	24	168.4	168.6	168.4
5	84.1	83.3	84.0	14	28.6	28.4	28.7	25	20.7	20.8	20.8
6	202.7	203.1	202.8	15	28.2	28.3	28.6	26	25.6	25.5	25.3
7	47.0	47.3	47.0	16	63.6	62.8	29.4	27	49.2	48.9	49.1
8	135.7	136.0	136.2	17	66.3	65.8	75.0	28	83.6	84.3	84.0
8a	132.9	132.8	133.2	18	58.6	60.2	147.4	29	29.1	28.8	28.8
9	179.3	179.2	179.2	19	18.7	18.8	110.9	30	30.3	30.3	30.6

5.23 (m, H-12), 1.80 (s, H_3-14), 1.75 (s, H_3-15), 2.55 (m, H_2-16), 4.43 (m, H-17), 1.05 (s, H_3-19), 1.38 (s, H_3-20)], and the third isoprenyl group which formed a CH-CH_2-CH-CH=C system [δ_H 2.60 (br d, J = 9.6, H-22), 2.36 (d, J = 12.8, Ha-21), 1.60 (dd, J = 9.6 and 12.8, Hb-21), and 7.49 (s, H-8)]. H-H COSY, NOE-difference and NOESY spectra (Fig. 2) were also recorded from the dilute solution. When the H-2 proton (δ_H 6.05)] was irradiated, an enhancement of the chelated hydroxy proton signal was observed which meant that the chelated hydroxy group (δ_H 12.53, OH-1) was *meta* to the another hydroxy group (δ_H 6.25, OH-3), and one isoprenyl group was at C-4 in the phloroglucinol aromatic ring. A NOESY correlation between H-14 and H-17 enabled us to determine the structure of **12**, which was a 7-methoxy derivative of **11** (Fig. 2).

It is of chemotaxonomic interest that polyprenylated xanthonoids were dominant only in *Garcinia* species. *Garcinia gaudichaudii* furnished as many as 15 such caged tri- and tetra-prenylated xanthonoids, i.e. gaudichaudic acids (**3**, **4**, **5a**, **5b** and **6**), gaudichaudiones (**1a**, **1b**, **2a**, **2b**, **7**, **8**, **9**, **10** and **11**) and 7-methoxygaudichaudione G (**12**). Gaudichaudiones A (**9**), B (**2b**), C (**10**) and D (**1b**) have been described previously[2] but **12** is interesting due to the oxygenated bridgehead. Polyisoprenylated xanthonoids are considered to have a mixed shikimate-triacetate and isoprenoid biosynthetic origin[3], the isolation of **12** is indicative that the prenylation of the shikimate-triacetate precursor has occurred prior to cyclisation to the caged system. The isolation of diastereomers for some of the above structures indicates there may be a lack of stereospecificity in the creation of one of the centres of chirality but the absolute configurations of such compounds have not yet been determined. Other chiral bicyclic and tricyclic prenylated xanthonoids have been reported from *G. morella*, *G. hanburyi* and *G. forbesii*[2-10] and the natural products can contain three to five isoprenyl groups.

Several of the present compounds (**1 - 6**, **8 - 10**) were evaluated for their cytotoxic effects against a panel of cancer cell lines. The tested compounds were broadly cytotoxic with ED_{50} values mostly in the range of 0.50 - 8 μg/ml; all compounds were cytotoxic except for one cell line where compounds **4** and **5a** were inactive. The aldehydic compounds in general show higher toxicity than the acids. Most planar xanthones do not show such bioactivity and the cytotoxicity of the present compounds appear to be facilitated by the tricyclo-4-oxa[4.3.1.$0^{3,7}$]decan-2-one moiety of each molecule.

Table 6. Cytotoxic Activity of Caged Prenylated Xanthonoids

Cell-line	1a	1b	2a	2b[2]	3	4	5a	6	8	9[2]	10
P388	0.20	0.32	0.38	0.51	2.8	15	11	3.5	0.24	0.42	1.2
WEHI1640	0.43	0.41	1.0	0.42	4.0	5.0	8.6	-	0.45	2.85	0.50
MOLT4	0.38	0.10	0.40	0.38	0.7	4.0	3.1	3.9	0.37	1.3	0.21
HePG2	3.8	3.5	6.0	3.2	9.2	>30	>30	8.0	4.1	8.0	20
LL/2	3.8	2.9	3.2	3.5	0.5	22	3.1	2.9	3.3	0.50	4.0

EXPERIMENTAL

General procedures. EIMS was run on a Micromass VG 7035 mass spectrometer at 70 ev. NMR spectra were recorded by Bruker ACF 300 [300 MHz (^{1}H) and 75 MHz (^{13}C)] and AMX 500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] instruments using $CDCl_3$, with TMS as an internal standard unless otherwise stated. IR spectra were recorded on a Bio-Rad FTIR spectrometer and UV spectra were recorded on a Hewlett Packard 8452A diode array spectrometer. Liquid chromatography was performed on silica gel (Kieselgel 60, 0.040-0.063 mm) and Sephadex LH-20. TLC was by Merck silica gel 60 F_{254} precoated glass plates.

Plant material. The leaves of *Garcinia gaudichaudii* were collected from Mt. Tawai, Kinabatangan, Sabah, Malaysia in 1996 and identified by J. T. Pereira and L. Madani. A voucher specimen SAN 135145 was deposited at the herbarium of the Forest Research Centre, Sepilok, Sandakan, Sabah, Malaysia. The dried and powdered leaves (1 Kg) were extracted first with hexane (24 h, 5 x 6 L), then with $CHCl_3$ (24 h, 5 x 6 L), and finally with acetone (24 h, 5 x 6 L) in a Soxhlet apparatus. The $CHCl_3$ yielded residue of 30 g *in vacuo* and this was fractionated by silica gel (Merck 9385, 1.8 Kg) column eluting with hexane, with a gradient of ethyl acetate to 100%, followed

by $CHCl_3$/MeOH (10:1 to 1:1 gradient). The compounds were eluted in the following order: **2a** (8.3 mg, 0.00083%), **1a** (8.9 mg, 0.00089%), **3** (34 mg, 0.0034%), **4** (6.2 mg, 0.00062%). From the crude hexane extract (14 g), **5a** (3.5 mg, 0.00035%), **5b** (1.4 mg, 0.00014%) and **6** (1.7 mg, 0.00017%) were isolated by silica gel (800 g) chromatography, eluenting with hexane:ethyl acetate (20:1) followed by $CHCl_3$:MeOH (1:1).

Gaudichaudione E (**1a**): yellow oil; $[\alpha]_D^{25}$ -436.2° (*c* 0.89, $CHCl_3$); UV (EtOH) λ_{max} 236 (4.06), 356 (3.96) nm; IR (KBr) ν_{max} 3454, 2974, 2923, 1739, 1689, 1635, 1457, 1334 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) data, Tables 1 and 2 respectively; EIMS *m/z* 562 $[M]^+$ (20), 544 (18), 501 (10), 423 (60), 382 (40), 305 (28%); HREIMS *m/z* 562.2573 (calcd for $C_{33}H_{38}O_8$, 562.2567).

Gaudichaudione F (**2a**): yellow oil; $[\alpha]_D^{25}$ -469.6° (*c* 0.83, $CHCl_3$); UV (EtOH) λ_{max} 232 (log ε 4.32), 358 (4.10) nm; IR (KBr) ν_{max} 3458, 2921, 2860, 1733, 1687, 1640, 1463, 1216 cm^{-1}; ^1H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) data, Tables 1 and 2 respectively; EIMS *m/z* 562 $[M]^+$ (10), 544 (40), 501 (24), 492 (70), 463 (80), 405 (60); HREIMS *m/z* 562.2574 (calcd for $C_{33}H_{38}O_8$, 562.2567).

Gaudichaudiic acid A (**3**): yellow oil; $[\alpha]_D^{25}$ -281.7° (*c* 3.41, $CHCl_3$); UV (EtOH) λ_{max} 234 (4.13), 354 (4.05) nm; IR (KBr) ν_{max} 3443, 2979, 2927, 1736, 1665, 1625, 1605, 1435, 1134 cm^{-1}; ^1H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) data, Tables 1 and 2 respectively; EIMS *m/z* 578 $[M]^+$ (1), 560 (2), 532 (3), 507 (6), 479 (6.5); HREIMS, *m/z* 578.2545 (calcd for $C_{33}H_{38}O_9$ 578.2516).

Gaudichaudiic acid B (**4**): yellow oil; $[\alpha]_D^{25}$ -325.5° (*c* 0.62, $CHCl_3$); UV (EtOH) λ_{max} 228 (4.20), 358 (3.97) nm; IR (KBr) ν_{max} 3449, 2979, 2926, 1736, 1684, 1632, 1449, 1331 cm^{-1}; ^1H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) data, Tables 1 and 2 respectively; EIMS *m/z* 578 $[M]^+$ (1), 560 (1), 532 (1), 505 (3), 479 (6.5); HREIMS, *m/z* 578.2518 (calcd for $C_{33}H_{38}O_9$, 578.2516).

Gaudichaudiic acid C (**5a**): yellow oil; $[\alpha]_D^{25}$ -355.7° (*c* 0.35, $CHCl_3$); UV (EtOH) λ_{max} 234 (4.19), 276 (4.10), 286 (4.06), 352 (3.91) nm; IR (KBr) ν_{max} 3447, 2963, 1654, 1593, 1458, 1262, 1135 cm^{-1}; ^1H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) data,Tables 4 and 5 respectively; EIMS *m/z* 592 $[M]^+$ (2), 574 (3), 559 (5), 521 (9), 435 (28), 349 (13), 247 (21), 149 (15); HREIMS m/z 592.2281 (calcd for $C_{33}H_{36}O_{10}$, 592.2308).

Gaudichaudiic acid D (**5b**): yellow oil; $[\alpha]_D^{25}$ -254.3° (*c* 0.14, $CHCl_3$); UV (EtOH) λ_{max} 222 (4.09), 276 (3.90), 286 (3.91), 350 (3.80) nm; IR (KBr) ν_{max} 3448, 2918, 1653, 1458, 1262, 1145 cm-1; ^1H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) data, Tables 4 and 5 respectively; EIMS *m/z* 592 $[M]^+$ (2), 574 (8), 559 (8), 521 (15), 503(6), 419 (50), 323 (19), 245 (28), 149 (29); HREIMS *m/z* 592.2305 (calcd for $C_{33}H_{36}O_{10}$, 592.2308).

Gaudichaudiic acid E (**6**): yellow oil; $[\alpha]_D^{25}$ -160.1° (*c* 0.143, $CHCl_3$); UV (EtOH) λ_{max} 220 (4.01), 278 (3.76), 286 (3.79), 358 (3.70) nm; IR (KBr) ν_{max} 3443, 2918, 1701, 1685, 1657, 1638, 1542, 1458, 1139 cm^{-1}; ^1H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) data, Tables 4 and 5 respectively; EIMS *m/z* 576 $[M]^+$ (3), 558 (8), 505 (100), 477 (68), 419 (40), 339 (40), 231 (50), 149 (25); HREIMS *m/z* 576.2365 (calcd for $C_{33}H_{36}O_9$, 576.2360).

Gaudichaudione G (**8**): yellow oil; $[\alpha]_D^{25}$ -197.9° (*c* 0.93, $CHCl_3$); UV (EtOH) λ_{max} 220 (4.32), 332 sh (3.85), 356 (3.93) nm; IR (KBr) ν_{max} 3454, 1752, 1654, 1646, 1637 cm^{-1}; ^1H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) data, Tables 4 and 2 respectively; EIMS *m/z* 562 $[M]^+$ (6), 534 (2), 519 (2), 507 (4), 479 (4), 367 (14), 325 (10), 229 (10), 43 (100); HREIMS *m/z* 562.2575 (calcd for $C_{33}H_{38}O_8$, 562.2567).

Gaudichaudione H (**12**): yellow oil; $[\alpha]_D^{25}$ -132.8° (*c* 0.33, $CHCl_3$); UV (EtOH) λ_{max} 214 (4.05), 332 sh (3.60), 358 (3.67) nm; IR (KBr) ν_{max} 3454, 1744, 1653, 1559 cm^{-1}; ^1H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) data, Tables 4 and 2 respectively; EIMS *m/z* 494 $[M]^+$ (1), 466 (78), 397 (60), 369 (40), 247 (62); HREIMS *m/z* 494.2312 (calcd for $C_{29}H_{34}O_7$, 494.2305).

Gaudichaudiones A-D (**9**, **2b**, **10**, and **1b** respectively) have been described earlier.[2]

Bioassays. The following cell lines were used: P388 (mouse lymphocytic leukemia), WEHI1640 (mouse fibrosarcoma), THP-1 (human monocytic leukemia), MOLT4 (human lymphoblastic leukemia), HePG2 (human hepatocellular carcinoma), LL/2 (Lewis lung carcinoma, mouse). Cell survival was evaluated by using MTT-tetrazolium assay as described previously.[16] Results are given in Table 4; according to the criterion set by the National Cancer Institute, USA, ED_{50} values of less than 30 μg/ml are considered cytotoxic.[17]

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